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A COMPARATIVE STUDY OF THE NET METABOLIC BENEFITS DERIVED FROM THE UPTAKE AND RELEASE OF FREE AMINO ACIDS BY MARINE INVERTEBRATES[1]

JOHN C. FERGUSON

Dept. of Biology, Eckerd College, Box 12560, St. Petersburg, Florida 33733

Abstract

A comparative study was undertaken to evaluate the overall significance of free amino acid (FAA) uptake and release in the biology of 21 species of marine invertebrates, representing 7 phyla. Measurements of transport fluxes were made in defined media using the fluorescamine method, corrected for ammonia excretion, and related to measured metabolic rates and natural environmental levels. Except for arthropods, in which net uptake was negligible, all the animals exhibited influx of FAA at rates dependent on media levels, and low, constant efflux. Equilibrium concentrations were always found to be less than the mean minimum bay water concentrations. Thus, all must derive benefit to commonly support at least 3–10% of their metabolism—approximately sufficient to provide for the needs of epidermal and associated tissues, including ciliary pumping over gills, tube feet activity, *etc.* Certain forms, including a sand dollar and brittlestar, appear to have become particularly adapted to make use of this resource, which may provide them more sustenance than ingested food. Yet other species support lesser, but still substantial, proportions of their metabolic requirements through these mechanisms. It is concluded that net uptake of FAA is an essentially universal property of soft-bodied marine invertebrates, and it plays an important part in their well-being, especially by providing for much of the energy needs of superficial structures and aiding in metabolite retention.

Introduction

Many estuarine and marine animals can obtain sustenance not only from the food they eat, but also through the direct assimilation of dissolved organic compounds found in their environments. This fact was originally demonstrated for a large group of diverse soft-bodied species by Stephens and Schinske (1961), although the original supposition was advanced much earlier, by Pütter (1909, 1911). Pütter, however, overstated his case and was largely discredited by the critical review of Krogh (1931) (*cf.* Jørgensen, 1976).

The actual significance of Stephens and Schinske's (1961) observations has also been brought into legitimate question. The concentrations of glycine they observed as being removed from solution were far in excess of normal sea water levels; the participation of micro-organisms could not be ruled out; the release of other amino acids from the animals to perhaps balance glycine uptake was not adequately investigated; and, there was no way of knowing where on the animals the uptake had really taken place. In short, it was not clear whether the observations they

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reported were a relatively insignificant artifact of the experimental conditions or an indication of an important physiological or ecological phenomenon.

In the last twenty years dozens of reports by a number of different investigators, employing much more elegant techniques, have succeeded in clarifying many of these uncertainties. Uptake of free amino acids (FAA), sugars, and fatty acids from natural concentrations can definitely occur, and its demonstration is, in most cases, little biased by micro-organisms. It is also apparent that similar compounds may be released back into sea water, but usually in comparatively small quantities, and that the uptake proceeds mainly into the exposed epidermal surfaces and not the gut. This body of work has been reviewed by Stephens (1968, 1972), Jørgensen (1976), and Stewart (1979); more recently summarized by Southward *et al.* (1979), Jørgensen (1980), Ferguson (1980a, b), and Stewart (1981); and evaluated in an American Society of Zoologists symposium (Seattle, 1980).

Clearly, the observed uptake of dissolved organics by estuarine and marine invertebrates is not an experimental artifact, but a real physiological process, which must provide advantages to these animals. It is still not clear, however, just what those advantages might be. Most marine animals eat, and by eating would seem to satisfy the bulk of their nutritional needs. Ferguson (1967, 1970), after autoradiographic studies on starfish, suggested that the benefit may not be so much to the whole animal as specifically to the epidermis: periodic eating would serve the nutritional needs of the internal parts, while continuous epidermal uptake would largely provide for the needs of this isolated tissue. A later study, (Ferguson, 1980a) confirmed in one species (*Echinaster*) that the amount of exogenous uptake normally occurring was about adequate to support the epidermal demands, but other experiments (Ferguson, 1980b) failed to show that the epidermis was limited to this source of nutrition: animals were found to be able to survive prolonged periods in near nutrient-free water.

What role, then, is served by exogenous uptake of dissolved organic compounds? This certainly is one of the major physiological and ecological questions remaining in the understanding of the biology of estuarine and marine organisms. The uptake may be a significant component of the total nutrition of some species, especially filter-feeders or smaller individuals. It may indeed be a vital source of sustenance for exposed soft tissues. It may be important in metabolite retention. It may be important in cell volume regulation or in other, as yet unknown, physiological activities. Or, it may be a vestigial relic from a more primitive state.

The full answer to this question may only come gradually, but clearly a first step towards the decipherment of the problem might be found in a modern comparative study which, by using newer, more sophisticated techniques, would go far beyond the basic findings of Stephens and Schinske (1961) in delineating the relative metabolic dependence of diverse species on this source of nutrition. The present account describes such data obtained from 21 species (of seven phyla) all examined within a relatively short period of essentially uniform environmental conditions.

Materials and Methods

The study was made feasible by the development and refinement of several procedures, which permitted the rapid accumulation of data with much greater sensitivity, accuracy, and facility than has previously been possible.

Sea water system

Accurate measurements of transport fluxes can only be made from a medium which is much purer than natural sea water, and if the animals and vessels used

are well flushed with this medium before the initiation of measurements. Freshly formulated artificial media have proven inadequate for this purpose because their quality is difficult to control and large volumes are needed. Therefore, a recirculating system was developed, which has proven invaluable for fulfilling this need. The system employs biological filtration on aged, granular, activated charcoal, multiple levels of mechanical filtration, oxidative reduction with ozone and UV light, and high levels of aeration. It reduces measurable organics to about 1% of the level of natural sea water. While it has been described briefly previously (Ferguson, 1980a, b), it is pictured more fully in Figure 1. The actual configuration does not appear to be critical except that efficient filtration (Millepore AP20 depth filters or equivalent) must occur both before and after ozonation, and the level of ozone must be adequate, but less than 0.05 mg/l in the final product water. Ozone was supplied from a generator of the design of Honn *et al.* (1976). The major mechanisms of organic removal are believed to be by micro-organisms on the biological filters, adsorption to chemical precipitates in the ozonation chambers, and oxidation. The original water is formulated from commercial synthetic sea salts mixed separately in batches, and added periodically to replace amounts withdrawn for various purposes. The temperature and salinity of the water are carefully adjusted to match that of the environment from which the animals are taken. The system can reduce fluorescamine reactants (measured as glycine) to less than 2×10^{-8} *M*. Healthy animals have been maintained in the water for over six months.

The adjustable syringe pump (SP) included in the system allowed the introduction of defined levels of amino acids to the water before it flowed into experimental vessels containing test animals. The amino acid mixture used for this purpose consisted of 509 mg "Eagle" (tissue culture) minimal amino acids (Difco), 80 mg "Eagle" non-essential amino acids (Difco), and 5 g glycine per liter of stock solution.

Chemical analyses

Amino acid levels were measured by an improved version of the fluorescamine method originally developed by Undenfriend *et al.* (1972). Borate buffer (pH 9.2; 0.5 *M*) was purified by filtration through a 0.45μ membrane filter, and 0.5 ml portions placed in a series of paired, carefully cleaned fluorometer tubes. The first pair of tubes then received 2.5 ml distilled water as a blank, and the second and third pairs similar amounts of 1×10^{-7} and 1×10^{-6} *M* freshly prepared glycine standards. As physical adsorption onto glass can be a problem at lower concentrations of standards, a minimal quantity of the detergent, Brij-35, was added to them, and a consistent pipetting procedure utilizing repeated rinsings was employed. With these precautions, linearity of fluorescence can be obtained.

Experimental samples were withdrawn after several pipette flushings with a 10 ml oral pipette, and 2.5 ml portions added to each of a pair of the prepared tubes (the remainder in the pipette was returned immediately to the experimental vessel). Within a few minutes of collecting the first samples, the blanks, standards, and samples each received 0.5 ml fluorescamine reagent (20 mg/100 ml acetone) injected vigorously with a mechanical pipette and immediately vortexed. After about ten minutes all the tubes were read against the distilled water blanks on a Turner 110 fluorometer equipped with a temperature stabilized door, "7-60" primary and "3" secondary filters, and usually a 3 or 10 × slit and a 10% neutral density filter. Care was maintained to prepare and read subsequent samples of each run with the same time sequence. Concentrations of unknown samples were calculated from the linear slope provided by the two sets of glycine standards.

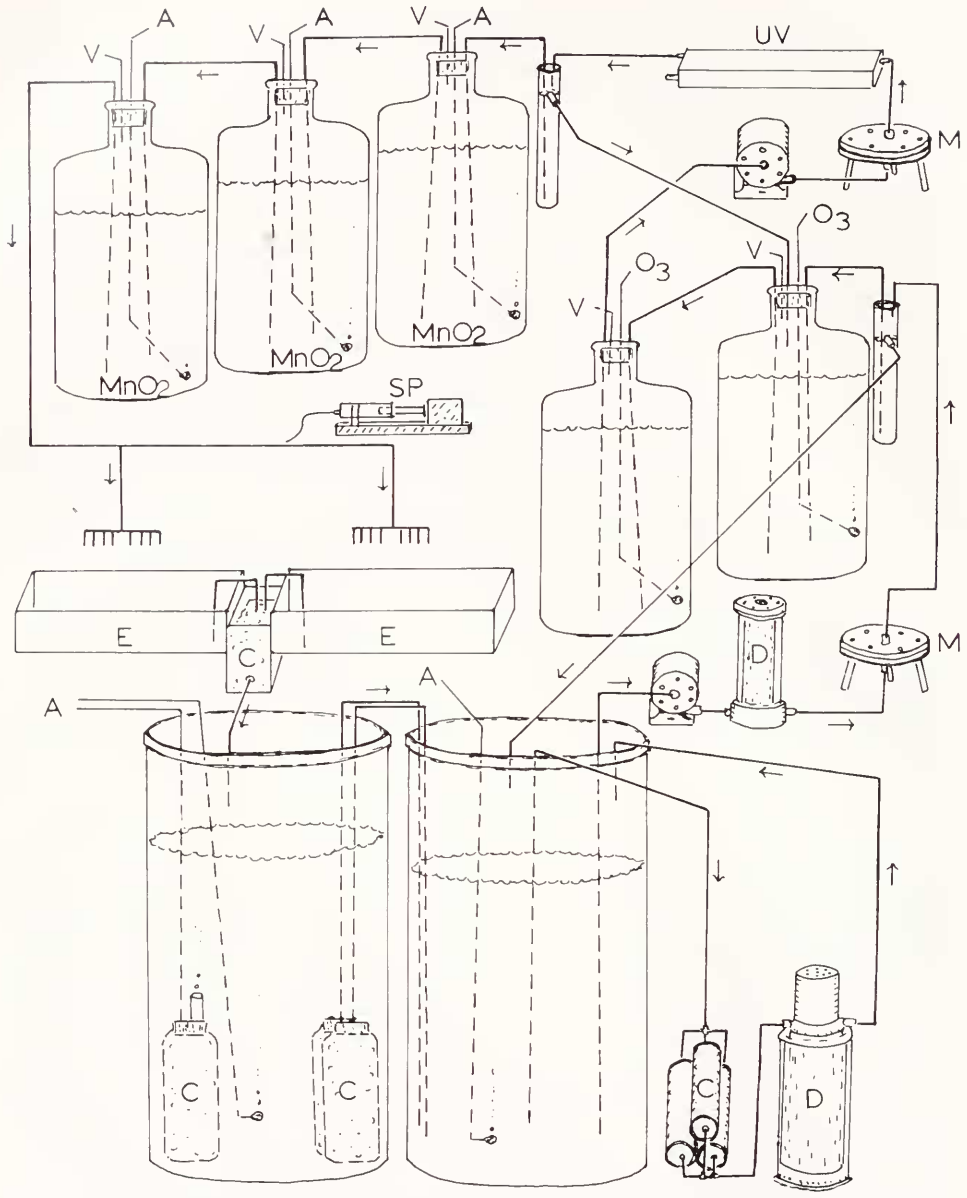

FIGURE 1. Recirculating system for removing organics from sea water media. A, air bubbler, C, granular charcoal filter; D, diatomaceous earth filter; E, experimental platform; M, Millipore depth filter; MnO_2, granular manganese dioxide on bottom of vessel to help breakdown ozone; O_3, ozone bubbler; SP syringe pump for re-introducing FAA; UV, ultraviolet irradiator; V, vent (to sewer drain).

Since the experimental samples were all taken from highly purified water, no preliminary cleaning treatment was necessary for them. Comparative samples of natural bay water, however, were filtered in the field using 0.45μ syringe mounted filters, or filtered (to the same degree) in bulk after returning them to the laboratory.

The fluorescamine procedure is largely specific for primary amines, but ammonia is also known to give a minor response, which probably varies with the details of the analytical procedure used. Jørgensen (1980) has reported a relatively elaborate formula to correct for it. In the present case, ammonium chloride standards produced a mean molar response of only 0.42% that of glycine, and this value has been used to correct measurements on the basis of their estimated ammonia content. In most cases the correction was a rather minor one.

Ammonia excretion was measured in separate metabolic experiments using the method of Solórzano (1969). Duplicate analyses were made on 5 ml samples read against distilled water blanks and ammonium chloride standards at 640 mμ on a Spectronic-20 colorimeter.

Ozone in the product water of the sea water system was monitored using "standard methods" (Taras *et al.*, 1971).

Animals

All the specimens were collected at low tide from the shallow waters of Tampa Bay, and identified according to the most authoritative published sources that could be obtained. Before use they were maintained one to three days in the laboratory in clean water of similar temperature and salinity to that from which they were obtained (approximately 34‰ and 29°C). Only healthy appearing, active animals were employed. The shells of molluscs were meticulously scrubbed with a brush, and tubiculous annelids carefully dissected from their tubes before the experiments. The hydroid, *Hydractinia*, was left attached to the shell on which it was found, and the measurements include the weight of this shell.

Metabolic measurements

Since the species used varied considerably in their weight and composition, it was necessary to find a meaningful common denominator by which they could be equated. Their metabolic rates, as measured through oxygen consumption, were chosen for this purpose. These rates were determined by placing one or more specimens in stoppered glass jars submerged in a container of purified sea water. The size of these jars was chosen so that usually within one to two hours the animals could reduce the oxygen content to about ⅔ of the initial level, at which time the experiment would be terminated. The jars also contained a rotating magnetic stirring bar protected in a small plastic cage. Small samples of water were withdrawn with a 2 ml syringe and injected into a Radiometer blood gas analyzer. The oxygen partial pressure values obtained were converted to volume measurements using solubility coefficients calculated from the formula provided by Green and Carritt (1967).

Also during these experiments, ammonia excretion rates were determined. Preliminary duplicate water samples were withdrawn from the jars just before they were sealed, and other samples taken immediately after the last oxygen measurement. The estimated excretion rates were calculated from the differences between the two sets of samples.

FAA exchange measurements

Animals, individually or in small groups, were placed in vessels scaled to their size (mostly less than one liter) and flushed with test media for 20 to 30 minutes at flow rates of several hundred ml per minute. A glass tube provided a steady

stream of gentle bubbles for mixing and some aeration. An experiment was begun by simultaneously taking the first samples and removing the inflow of flushing medium. Subsequent samples were withdrawn after 2, 5, 10, 15, 20, 30, 40, and 60 minutes. (In some cases with small volumes the 2 and 15 minute samples were omitted). Each experiment was repeated three times with the same animals; first at minimal initial FAA levels, then (by adjusting the flow rate of the syringe pump) at an intermediate level estimated to be within the range of natural sea water, and finally at a level judged to be slightly higher than the natural concentration. Thus, 27 (or 21) datum points, each the mean of a duplicate analysis, were obtained to characterize the rates of FAA uptake and release by each species.

Results

An example of the results of the FAA experiments may be seen in Figure 2. Each of the illustrated datum points represents the measured value less a minor correction (lower, double-dashed line) for estimated fluorescamine response due to ammonia released by the specimens (upper, dashed line).

To practically evaluate these data and meaningfully compare different animals, the curves must be reduced to simpler parameters. A relatively straightforward empirical mathematical model appears to provide the most convenient and effective procedure for doing this, rather than models based solely on enzyme kinetic theory (Ferguson, 1980a). This model assumes that the rate of uptake by the animals is dependent on the FAA concentration of the medium, and that the rate of release, within the time frame and conditions of the experiment, is constant. Thus, the change of concentration of FAA within the experimental vessel is described by:

$$\frac{dC_t}{dt} = \lambda - kC_t$$

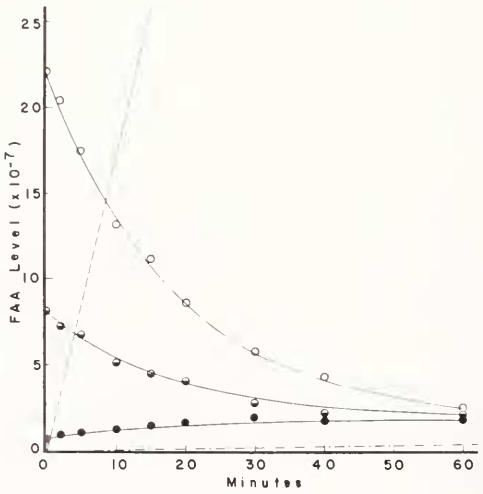

FIGURE 2. Changes in media concentration of FAA produced with three different initial levels (represented by ○, ◒, and ●) by the sea urchin, *Lytechinus variegatus* (81.0 g). The curved lines represent values predicted from the model ($k = 6.63 \times 10^{-4}$; $\lambda = 12.35 \times 10^{-11}$ moles/g/min.). Also shown is the estimated accumulation of ammonia from excretion (upper, dashed straight line), and the fluorescamine response correction based on it (lower, double-dashed straight line) (Initial fluid volume = 1025 ml).

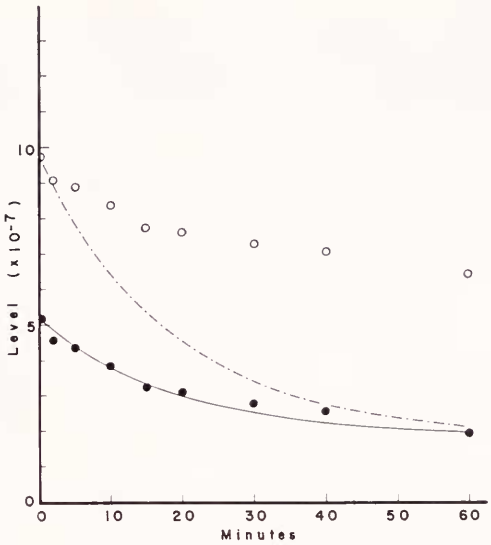

FIGURE 3. Removal of primary amines from filtered bay water by *Lytechinus*. Open symbols represent actual measured changes in concentration; closed symbols represent values less a 4.5×10^{-7} *M* correction for non-FAA reactants. Curved lines are the changes predicted by the model (same parameters, λ and k, as in Fig. 2)—dashed line with the uncorrected concentration; solid line with the corrected concentration. With the correction, a much improved fit is obtained.

where (with standardization of units),

λ = the rate of FAA efflux from the animals (moles/g/min.)

k = the fractional rate of influx (from 1 liter into 1 g/min.)

C_t = the FAA concentration (*M*) of the medium at time "t" (min.).

The working form of the equation, including corrections for specimen weight (w) (=g) and vessel fluid volume (v) (=ml) is:

$$C_t = \frac{\lambda}{k} + \left(C_0 - \frac{\lambda}{k} \right) e^{-k \cdot t \cdot w \cdot 1000/v}$$

It may be further noted that the ratio of the two constants (λ/k) corresponds to the equilibrium concentration of FAA in the medium at which the rate of influx would be just equal the rate of efflux.

The major difficulty in the practical use of this equation is that it must be solved by inspection. In the present case, this was facilitated by a computer program, which also corrected for the small volume changes induced by the withdrawal of each sample, and the ammonia effect. The values of k and λ selected were those which produced a correlation coefficient (r) closest to unity when predicted values were matched to the 27 (or 21) datum points.

The curved lines shown in Figure 2, then, represent the best fit values predicted by the model in this fashion. Table I lists the determined values for the fractional rate of influx (k), rate of efflux (λ), and related data recorded for each of the 21 species. The computed equilibrium levels (λ/k) are listed in Table II.

TABLE I

Influx and efflux of free amino acids (FAA) by various estuarine species

Species (number)	Total weight (g)	FAA influx (k) ($\times 10^{-4}$)	FAA efflux (λ) (10^{-11} m/g/min.)	Fit (r)	NH_3 Excretion rate (10^{-11} m/g/min.)
PORIFERA					
Cliona celata (1)	315.0	1.39	0.70	0.99176	373
CNIDARIA					
Hydractinia echinata (on shell)	10.7	5.03	14.70	0.99939*	408
Calliactis tricolor (1)	2.0	6.91	22.35	0.99712*	0
MOLLUSCA					
Fasciolaria distans (2)	71.0	1.52	4.22	0.99935	138
Melongena corona (1)	112.5	1.86	4.00	0.99827	303
Cantharus tinctus (9)	33.5	3.22	9.90	0.99861	718
Macrocallista nimbosa (1)	165.0	1.75	3.90	0.99742	42
Mercenaria campechiensis (1)	286.0	0.69	0.83	0.99949	193
Crassostrea virginica (1)	102.0	2.27	6.15	0.99642	185
Argopecten irradians (1)	21.4	11.72	15.95	0.99570	678
ANNELIDA					
Glycera americana (3)	0.8	15.43	61.00	0.99892*	933
Diopatra cuprea (9)	4.5	8.49	54.80	0.99674	2867
Cirratulus hedgepethi (2)	3.6	7.29	42.00	0.99802*	712
ARTHROPODA					
Limulus polyphemus (1)	10.9	1.34	0.00	0.99967	3090
Emerita talpoida (6)	8.0	2.97	29.60	0.99951	1200
ECHINODERMATA					
Astropecten articulatus (1)	18.5	2.76	6.90	0.99959	61
Ophiophragmus filograneus (2)	1.9	7.15	18.90	0.99785*	43
Leptosynapta crassipatina (1)	0.3	6.05	41.00	0.99981*	381
Lytechinus variegatus (1)	128.5	6.63	12.35	0.99914	833
Mellita quinquiesperforata (1)	69.0	2.56	4.75	0.99953	300
CHORDATA					
Styela plicata (1)	59.8	2.35	1.78	0.99620	417

(m = moles)

Best fit of 27 or 21 (*) points after correcting for NH_3 excretion (34‰ S, 29°C).

The values of k and λ are useful for some purposes, but since they are based on weight, they are highly affected by the relative proportion of skeleton, body fluid, and active tissue of each species. Thus, a somewhat more meaningful comparison is obtained if influx and efflux are computed in terms of metabolic rate. The measured metabolic rates and the proportional FAA fractional influx and efflux calculated in terms of them are presented in Table II.

TABLE II

Comparison of free amino acid influxes and effluxes per unit metabolic rate, and equilibrium levels (normal environment = 7.5 to 17 × 10^{-7} M FAA).

Species	Metabolic rate (μL O_2/g/min.)	FAA influx (10^{-4} M/μL O_2/min.)	FAA efflux (10^{-11} M/μL O_2/min.)	FAA equilibrium level (10^{-7} M)	FAA sustaining level (10^{-7} M)
PORIFERA					
Cliona celata	0.25	5.6	2.8	0.50	181
CNIDARIA					
Hydractinia echinata	0.60	8.4	24.5	2.62	123
Calliactis tricolor	0.62	11.1	36.0	3.61	93
MOLLUSCA					
Fasciolaria distans	0.60	2.5	7.0	2.77	398
Melongena corona	0.78	2.4	5.1	2.15	422
Cantharus tinctus	0.45	7.2	22.0	3.07	143
Macrocallista nimbosa	0.31	5.6	12.6	2.22	180
Mercenaria campechiensis	0.11	6.3	7.5	1.20	161
Crassostrea virginica	0.20	11.4	30.8	2.70	91
Argopecten irradians	0.97	12.1	16.4	1.36	85
ANNELIDA					
Glycera americana	1.57	9.8	38.9	3.95	106
Diopatra cuprea	2.38	3.6	23.0	6.45	287
Cirratulus hedgepethi	0.98	7.4	42.9	5.76	141
ARTHROPODA					
Limulus polyphemus	9.77	0.1	0.0	0.00	7300
Emerita talpoida	4.68	0.6	6.3	9.96	1590
ECHINODERMATA					
Astropecten articulatus	0.46	6.0	15.0	2.50	170
Ophiophragmus filograneus	0.54	13.2	35.0	2.64	79
Leptosynapta crassipatina	2.12	2.9	19.3	6.77	358
Lytechinus variegatus	0.27	24.6	45.7	1.86	43
Mellita quinquiesperforata	0.15	17.1	31.7	1.85	60
CHORDATA					
Styela plicata	0.60	3.9	3.0	0.75	257

While the values listed are characteristic of each species under the conditions that have been defined, it is particularly useful to relate them to the actual FAA levels that the animals encounter in their normal habitats, or at least, natural bay water. Therefore, samples of bay water were collected from the same vicinities as were the animals. These were immediately filtered in the field and analyzed within 30 minutes. They revealed a considerable variability and distinct differences between conditions at low tide (a few cm from the bottom) and high tide (about a meter above the bottom). The mean response of 12 low tide samples (± standard deviation) was 21.63 ± 6.40 × 10^{-7} M, while 13 high tide samples had a mean of 12.64 ± 2.23 × 10^{-7} M. Furthermore, it was found that if containers of bay water were returned to the laboratory and filtered in bulk, much lower levels still would be recorded. Six such samples had a mean of 7.98 ± 2.17 × 10^{-7} M. This decline was assumed to be due to the rapid removal of dissolved organics by the rich

plankton content. The overall appearance is that large quantities of FAA leach out of bottom detritus and sediments into the water column and are rapidly taken up by living organisms in the water.

All these measurements were, of course, recorded in glycine equivalents. Clearly the values do not represent just FAA, but also other filterable primary amines and, especially, ammonia. While the means were not available to differentiate these components, a simple experiment was conducted to evaluate the total level of non-FAA represented.

A quantity of bay water was brought to the laboratory and filtered as quickly as possible. The specimen of *Lytechinus* previously studied was then flushed with this water and the experiment repeated using the same water as the experimental medium. The results may be seen in Figure 3. The measured levels declined, but at a rate much slower than that predicted by the model using the constants determined from defined media. However, if a value of 4.5×10^{-7} *M*, representing metabolically inert fluorescamine reactants, is subtracted from the observed values, a close fit is obtained. The same result has been reached in similar experiments with other animals.

TABLE III

Percent of metabolism supported by free amino acid in the normal range of bay water concentrations (7.5 to 17 × 10^{-7} M).

Species	% Metabolism gross (uptake only)	% Metabolism net (uptake − loss)
PORIFERA		
Cliona celata	4.2–9.5	3.9–9.2
CNIDARIA		
Hydractinia echinata	6.3–14.2	3.8–11.8
Calliactis tricolor	8.4–18.9	4.8–15.3
MOLLUSCA		
Fasciolaria distans	1.9–4.3	1.2–3.6
Melongena corona	1.8–4.1	1.3–3.5
Cantharus tinctus	5.4–12.2	3.2–10.0
Macrocallista nimbosa	4.2–9.6	3.0–8.3
Mercenaria campechiensis	4.7–10.7	3.9–9.9
Crassostrea virginica	8.5–19.3	5.4–16.2
Argopecten irradians	9.1–20.5	7.4–18.9
ANNELIDA		
Glycera americana	7.4–16.7	3.5–12.8
Diopatra cuprea	2.7–6.1	0.4–3.8
Cirratulus hedgepethi	5.6–12.6	1.3–8.4
ARTHROPODA		
Limulus polyphemus	0.1–0.2	0.1–0.2
Emerita talpoida	0.5–1.1	(−)0.2–0.4
ECHINODERMATA		
Astropecten articulatus	4.5–10.2	3.0–8.7
Ophiophragmus filograneus	9.9–22.5	6.4–19.0
Leptosynapta crassipatina	2.1–4.8	0.2–2.9
Lytechinus variegatus	18.4–41.7	13.8–37.2
Mellita quinquiesperforata	12.8–29.0	9.6–25.8
CHORDATA		
Styela plicata	2.9–6.7	2.6–6.4

TABLE IV

*Comparison of observed rates of uptake and release of FAA by a sunray clam (*Macrocallista nimbosa*) while closed and while open with siphons extended.*

	Influx (k)	Efflux (λ)	Fit (r)	Metabolic rate
	($\times 10^{-4}$)	(10^{-11} m/g/min.)		(μL O_2/g/min.)
Closed	0.02	0.17	0.99968	0.03
Open	1.75	3.90	0.99742	0.31

This correction value of 4.5×10^{-7} *M* was then subtracted from the mean values of field filtered water samples. It was thus estimated that the mean Tampa Bay FAA levels vary from approximately 7.5 to 17×10^{-7} *M*, depending largely on the state of the tide. The extreme range of observed values, corrected in this fashion, was 4.0 to 31.3×10^{-7} *M*.

Using the mean values (7.5 to 17×10^{-7} *M*), and the constants, k and λ, determined for each species, it is possible to calculate the quantity of FAA taken up and given off within this range of environmental conditions. Furthermore, since the aerobic metabolism of a mole of amino acid (molecular weight 100) requires approximately 100 liters of oxygen, an estimation may be made of just what proportion of each species' metabolism is satisfied through dissolved FAA in the water. These computations have been tabulated in Table III.

The actual environmental levels encountered by some species, especially those living in the sediments, are difficult to evaluate, but they may be much higher than those of sea water. It is useful, then, to look at what implied effects higher concentrations might have in supplying the metabolic needs of various animals. Thus, a calculation has been made (using the same equivalency of 1 mole FAA to 100 liters O_2 consumption) to estimate the environmental level that would produce net influx just providing for the total measured metabolic rate. These values, referred to as "sustaining" levels, are also listed in Table II. Their presentation is for reference, and it is not meant to be implied that the animals can (or cannot) actually sustain themselves completely at these levels solely from FAA, or that the transport systems responsible for uptake would function with the same effectiveness at these higher concentrations. Indeed, saturation of the transport systems might be expected.

Finally, one additional experiment was completed to serve to some extent as a control for the other work. Strong rubber bands were placed around a previously studied sunray clam (*Macrocallista*), effectively preventing it from pumping. The complete experimental procedure was then repeated. As expected, the observed metabolic rate and FAA fluxes were reduced to negligible levels (Table IV).

DISCUSSION

The mathematical model used appeared to be wholly satisfactory for describing the data obtained in each experiment. The illustrated case of *Lytechinus* (Fig. 2) is fairly typical of the results obtained with the other animals. Any model, however, is a simplification of reality. The present approach has assumed a homogeneity of the amino acids taken up and released that obviously does not exist. A mixture of amino acids was used in the experiments in the belief that the data obtained by that means would be the most reflective of the unknown natural situation. The high

proportion of glycine was included not only because all measurements were made against this substance as a standard, but also because previous work (Ferguson, 1971) revealed that it is by far the most significant component released by starfishes into sea water.

The success of the model in fitting the data seems to generally bear out these assumptions. It also substantiates the implication of the model that each species, for the defined conditions, has a characteristic rate of uptake that is dependent primarily on the external FAA concentration. For this to be the case, the transport systems involved must possess high affinities and saturation levels. Such properties have been reported by other investigators, but their demonstration may be affected by the experimental conditions (*e.g.*, Wright and Stephens, 1977; Wright *et al.*, 1980). Since the model used in the present work is empirical, it makes allowance not only for the kinetic properties of the various transport systems, but also for the physical limitations of movement of media over the absorptive surface, and possible other biological characteristics of each species.

The model also implies a constant release rate from the organisms. Alternative models have been examined which allow the intracellular pools to increase with uptake, and thus induce greater efflux. These more complex models appeared to offer little or no advantage in matching the data, and they were abandoned. The intracellular amino acid pools seemed to remain rather stable and scarcely affected by the range of substrates employed in these experiments. Such stability could be due to their large size or to rapid metabolic interconversions within the cells.

Clearly there are major differences between the species in their characteristic fractional rates of influx and rates of efflux (Table I). Many of these differences must relate to the various physical conformations of the animals. For example, the southern quahog clam, *Mercenaria*, has low recorded rates, but most of its weight is represented by its especially massive shell. The errant polychaete, *Glycera*, on the other hand, has extremely high rates, but completely lacks a hard skeleton. There probably is no fully satisfactory way to evaluate the differences of such disparate species, but a number of calculations included in Tables II and III are helpful.

It can be seen immediately in these that only arthropods fail to carry on meaningful exchanges. This must be related to the presence of an exoskeleton covering all their exposed surfaces, and it was a basic finding of Stephens and Schinske (1961). In the present cases, minor values have been recorded, especially for the small and very active mole crabs (*Emerita*). This may possibly be due to epiflora, as Anderson and Stephens (1969) have suggested in studies with several small arthropod species. Bacterial uptake of hexoses has also been described by Castille and Lawrence (1979) in work with post larval penaeid shrimp. If the values listed here do reflect ingestion and excretion, these processes can only represent very minor proportions of the animals' total metabolic activities.

While some contribution by epiflora cannot be completely ruled out in other cases, the effect must be almost negligible. The animals were all clean and well flushed with nearly sterile media before the experiments, and the levels of organics used were not such as to encourage a rapid bloom of bacteria within the short time frames studied. Cultures of wipes taken from animals and vessels failed to reveal large amounts of bacterial growth and, in other work, antibiotics have not significantly inhibited uptake. Autoradiographs (Ferguson, 1967) have shown that uptake is into the epidermal layer, and not into particles attached on the surface. Furthermore, the control experiment with the bound specimen of *Macrocallista* (Table IV) is not indicative of a significant bacterial contribution in comparison to the normal exchanges that take place.

All of the species other than arthropods to demonstrate significant levels of uptake and net uptake, as well as specific differences. The equilibrium levels (Table II) are all low, and definitely less than the mean minimum FAA level (7.5×10^{-7} *M*) found in the waters from which the animals come. This confirms the hypothesis that soft-bodied marine animals do indeed gain net sustenance through the removal of dissolved organics from sea water, and the existence of this process should now be assumed to be universal for all such species, unless specific proof is acquired to the contrary. The only substantial report claiming the reverse process, that marine animals suffer net loss of amino acids, is that of Johannes *et al.* (1969) on the commensal flatworm, *Bdelloura*. As the newer methods appear to be considerably more sensitive and reliable than theirs, it would be appropriate for this species to be re-examined.

It is significant that mechanisms providing for net uptake of FAA are at least as well developed in sponges as in chordates. They exist as commonly in the lower phyla as in the higher ones. Thus, they must have evolved very early, probably in some form even before any of these phyla were differentiated. They possibly represent the most ancient means by which animals obtain their nutrition. While they have been lost with the development of impervious exoskeletons, as in the arthropods, their apparent universal retention by other invertebrate species attests to their continuing significance in the biology of these forms. Most of the differences in the values of the parameters listed in Tables I, II, and III, thus, appear to relate to specific evolutionary adaptations in life styles, and not to unique differences that developed phylogenetically.

A comparison of the influx and efflux rates in Table I shows that when one rate is high there is a tendency for the other to be high also and *vice versa*, and this is partly responsible for the consistency of the low equilibrium levels (Table II). Higher paired rates are especially evident for *Calliactis, Crassostrea, Argopecten, Ophiophragmus, Lytechinus,* and *Mellita*. Thus, there seems to be some kind of connection between the two. Most likely, influx and efflux occur in the same tissues, and the correlation reflects a measure of the amount of the metabolically active tissues that are exposed to efficient ventilation.

While the equilibrium levels for all the soft-bodied species are low, those of some are slightly higher than others. This is especially the case for the burrowing tubiculous annelids and, interestingly, the burrowing holothuroid, *Leptosynapta*. Stephens (1975), Costolpulos *et al.* (1979), and others have speculated that burrowing annelids can obtain significant FAA from high levels that appear to exist in the sediments, but since the animals normally live in tubular chambers formed from various types of secretions, it is uncertain just how much advantage they can take of this source, and their true relationship may be more with the water at the sediment-water interface. In the present study it was found that the near bottom water (low tide) itself contains significantly more FAA than that higher in the water column. So, the worms most certainly must be exposed to somewhat elevated FAA levels. Their higher equilibrium values appear to reflect an adaptation to these levels.

The molluscs as a group make for some interesting comparisons. In the case of the predacious gastropods, *Fasciolaria* and *Melongena*, benefit from exogenous uptake is limited to several percent of the metabolic rate (Table III). Presumably much of this uptake is located in the gills, as much of the rest of the body is covered by shell. While it is a small quantity, it may be sufficient to supply the energy needs of the ciliated cells that maintain the water flow over these organs to sustain respiratory exchange.

In contrast, the bay scallop, *Argopecten*, exhibits a much greater metabolic

dependence on FAA. This species not only has large gills in relation to its body size, it also has numerous pallial tentacles and an extensive area to its mantle. Thus, its adaptations include a rather large ventilated surface area and a reduced mass. Its relatively high dependence on FAA may be a factor in the restriction of its normal habitat to presumably richer bay and nearshore waters, especially on grass beds. The oyster, *Crassostrea*, while possessing a very different life style, appears to share some of these same properties.

Heavier, burrowing bivalves, such as *Macrocallista* and *Mercenaria*, obtain benefits from net uptake at a level somewhat intermediate between the predacious gastropods and the filibranchs. As filter feeders they need large gills and efficient pumping, but they appear to sustain their larger body masses more completely through ingested food. Again, their intermediate levels of uptake may largely serve the energy needs of the ciliary pumping process.

Other interesting cases of adaptations are seen in the echinoderms. *Lytechinus* has the highest net uptake, supporting up to 37% of its metabolic rate at the 17×10^{-7} *M* environmental level. This species normally hides itself under shells and weeds it picks up with its many long and active tube feet. While this behavior itself might have nutritional significance in enhancing the FAA levels in the vicinity of the animals, the high level of dependence definitely correlates with the activity of the tube feet and other appendages. Autoradiographs (Ferguson, 1967, and others) have shown that these structures are especially efficient in taking up dissolved organics. Thus again, the metabolic activities of the superficial structures appear to be maintained in very large part through the direct assimilation of nutrients from the environment. Also, sea urchins often pump water through their digestive tracts, presumably to assist in respiration, and uptake might occur internally *via* this route (*cf.*, Ferguson, 1969; Pearse and Pearse, 1973).

The sand dollar, *Mellita*, and the brittlestar, *Ophiophragmus*, also exhibit a very high utilization and metabolic dependence on FAA. Both these species live in the upper levels of sandy sediments and detritus—the former near high energy beaches, the latter on grass flats. Unlike the worms, their naked bodies and motility would fully expose their surfaces to the higher levels of FAA that undoubtedly occur in these substrates. Therefore, they must have even larger proportions of metabolic dependence on this nutrient source than is indicated by Table III. Especially intriguing are the low sustaining levels (Table II) calculated for both of these species. These are well within the natural substrate levels that could reasonably be expected to be encountered. Thus, it is highly probable that exogenous uptake of dissolved organics, and not feeding, actually represents the primary nutritional source of both these species. A similar conclusion was reached for the sand dollar, *Dendraster*, by Stephens *et al.* (1978).

While exogenous uptake of FAA may be the major nutritional source of these last two forms, it is undoubtedly not an exclusive one. There is no evidence that these or any of the other animals examined in this study can survive without feeding, and certainly evolution towards different feeding strategies has been a predominant feature of most metazoans. On the other hand, it is also apparent that these and other species have undergone considerable evolutionary adaptation to enhance the benefits they can derive from exogenous uptake mechanisms. Recognition of this fact can perhaps greatly assist in understanding the peculiar structural complexities of many types. The short spineous surface texture of sand dollars and brittlestars, the frilled mantle of some molluscs, and similar kinds of ornamentation on other soft-bodied species may all be adaptations to facilitate epidermal assimilation of dissolved organic substances. The pogonophorans, lacking a gut, may as a phylum

be even more highly adapted towards this source of nutrition (*cf.*, Southward *et al.*, 1979). Relatively little work has yet been completed on the exotic structural forms frequently found in the meroplankton, but this nutritional mechanism may be especially significant for larva, particularly those that utilize ciliary forms of locomotion (*cf.*, North, 1975; Fankboner and DeBurgh, 1978; Rice *et al.*, 1980).

The fact that most animals have retained mechanisms for both exogenous uptake of dissolved nutrients as well as feeding appears to indicate that each serves significant functions that are not readily provided by the other. That is, the two mechanisms are not so much redundant as complimentary. The levels of net utilization of FAA reported here, commonly less than 10% of the total metabolic needs, conforms with the previously developed hypothesis (Ferguson, 1967, 1970, 1980a,b) that this uptake generally supplies the nutritional needs of the epidermis and active superficial structures. Physiologically, this would appear to have at least two benefits. First, it would provide nutrition to parts of the body which are far removed from internal reservoirs. This process must have much greater facility than those associated with food collection, digestion, assimilation, storage, and translocation. It can provide energy directly to isolated parts, which in some cases (ciliary appendages, gills, tube feet, *etc.*) may have large energy demands. As an essentially continuous process of high efficiency, it is particularly effective in supporting metabolic activities which also tend to be continuous. Second, it assists in providing for the isolation of the internal environment from the external one. The natural levels of FAA are often orders of magnitude less than those maintained in the body of fluids of most marine animals (*cf.* Awapara, 1962). Clearly, for unprotected species these gradients create problems, especially if large quantities of metabolites must be conducted to the epidermal tissues to support ciliary movement, secretion, and other forms of work. If superficial tissues can obtain most of their sustenance directly from the environment, permeability barriers may develop to help retain these metabolites. Certainly the data reported here show that losses of FAA are very low indeed, and even these residual losses are more than compensated for by the uptake mechanisms.

It is concluded, then, that direct uptake of FAA (and probably other dissolved organic nutrients) plays a vital role in the biology of essentially all soft-bodied marine invertebrate species, especially serving to support epidermal functions and metabolite retention. Furthermore, a number of species have become specifically adapted to utilize this form of sustenance as a major source of nutrition with it in some cases providing more benefits than ingested food. Thus, the importance of these mechanisms should not be overlooked in either physiological or ecological investigations of marine animals.

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